/ETHANOL PRODUCTION FROM GRAIN DUSTS, BREAD WASTE, AND CAKE WASTE WITH AND WITHOUT BREWERS' CONDENSED SOLUBLES (BCS)/

by

CHUL-HO CHOI

B.E. Han-Yang University, Korea, 1980

A MASTER'S THESIS

submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

in

FOOD SCIENCE

Department of Agricultural Engineering
KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

Approved by:

Major Professor

LD 3666 .T4 1986 056

TABLE OF CONTENTS

	Pag
INTRODUCTION	1
OBJECTIVES	3
REVIEW OF LITERATURE	3
Energy from grain alcohol	3
Historical perspective of fuel alcohol	
production	3
\Fuel economy of ethanol	5
*Energy savings with fuel alcohol	5
Biomass raw materials for ethanol production	9
Ethanol production from grain by-products	12
Small scale ethanol production	13
Basics of ethanol production	14
MATERIALS AND METHODS	20
Materials	20
Methods	21
Liquefaction and saccharification	22
Fermentation	23
RESULTS AND DISCUSSION	24
Proximate analysis	24
Fermentation of hydrolyzed grain by-products and	
their mixtures with BCS	24
Yield of ethanol	45

	Page
Amino nitrogen in hydrolyzed grain by-products	
and BCS	47
Cost analysis for ethanol production	50
CONCLUSIONS	60
LITERATURE CITED	62
ACKNOWLEDGMENTS	57

TABLES

			Ē	age
Table	1.	Comparison Between the Properties of Iso-octane and Ethanol		7
Table	2.	Biomass Resource Base for Ethanol Production in the U.S		10
Table	3.	Proximate Chemical Composition of Grain By-products and BCS (dry basis)		25
Table	4.	Average Sugar Composition in Saccharified Bread Waste and Cake Waste		25
Table	5.	Carbon Dioxide Production during Fermentation of Saccharified Grain Dusts and Their Hixtures with BCS		23
Table	6.	Ethanol Yields Produced by Yeast Fermentation of Saccharified Grain By-products and Their Mixtures with Glucoamylase-Treated BCS; Optimum Fermentation Times and Fermentation Efficiencies		35
Table	7.	Carbon Dioxide Production during Fermentation of Saccharified Bakery Wastes and Their Mixtures with BCS		36
Table	8.	Ethanol Yields and Optimum Fermentation Times Obtained from Yeast Fermentations of Enzyme-Digests of Grains Mixed with Glucoamylase-Treated BCS		40
Table	9.	Sugar Consumption and Ethanol Production during Fermentation of Cake Waste		41
Table	10.	Effect of pH on Fermentation of Saccharified Bread Waste and Cake Waste		44
Table	11.	Amino Nitrogen in Saccharified Grain By-products and BCS		43
Table	12.	Amino Nitrogen in Hydrolyzed Grain Materials and Their Optimum Fermentation Times		49
Table	13.	Time Required for Fermentation and Amino Nitrogen Content of Various Worts		51

			Page
Table	14.	Fermentation Times of Saccharified Grain Dusts and Their Mixtures with BCS Based on Equal Initial Glucose Contents (5.6% w.b.)	52
Table	15.	Estimated Feedstock Cost from Various Raw Materials and Their Mixtures with BCS	56
Table	16.	Costs for Ethanol Production from Various Feedstocks and Their Mixtures with BCS	57
Table	17.	Total Production Cost for Ethanol from Various Feedstocks and Their Mixtures with BCS	53
Table	18.	Total Production Cost - 1981 Basis 190 ^o Proof Alcohol from Corn	59

FIGURES

		pag
Figure 1.	Flow diagram of an ethanol production process	. 15
Figure 2.	Carbon dioxide production during fermentation of saccharified mixtures of wheat-corn dust and BCS. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.2	. 29
Figure 3.	Carbon dioxide production during fermentation of saccharified mixtures of corn-sorghum dust and BCS. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.2	. 31
Figure 4.	Carbon dioxide production during fermentation of saccharified mixtures of sorghum-soybean dust and BCS. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.2	. 33
Figure 5.	Carbon dioxide production during fermentation of saccharified mixtures of bakery wastes and BCS. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.6	. 37
Figure 5.	Sugar consumption and ethanol production during fermentation of cake waste. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.5	. 42
Figure 7.	Relationship between amino nitrogen inenzyme hydrolyzed substrates and their fermentation times measured by gasograph	. 53

INTRODUCT TON

The petroleum supply disruption of the 1979's and the resulting dramatic escalation of imported crude oil prices spurred substantial interest in the production of fuel alcohol from domestically abundant renewable resources (Gill and Allen 1985). During the past 6 years, production of fuel alcohol has expanded by almost 400% (USDA 1985a). Along with the promulgation of Environmental Protection Agency's (EPA) new regulation requiring a reduction in the lead content of gasoline from 1.1 g to 0.1 g per gal in January 1986, the demand for ethanol as an octane-enhancer is expected to pick up significantly (EPA 1935, Gill and Allen 1985).

Good quality convencional feedstocks have traditionally been used by the beverage alcohol industry. Although the beverage alcohol 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 (Fahrenholz 1983). It is also possible to use many types of grain by-products such as grain dusts, bakery wastes, and brewery wastes. Complete utilization of those by-products is both a necessity and a challenge. Furthermore, substantial savings could be obtained if those waste materials were utilized as feedstocks for fuel alcohol production.

Brewers' condensed solubles (BCS) is a mixture of the concentrated water-soluble and suspended by-products from the manufacture of beer. BCS is a rich source of fermentable carbohydrates and contains peptides, phosphorous, calcium, trace minerals, and some water-soluble vitamins (Sebree et al 1983).

Grain dust is always present in grain handling facilities, and it constitutes a fire, explosion, and health haz ard (Martin and Stephens 1977). Physical and biological characteristics of grain dust were determined by a number of investigators (Martin 1981, Martin and Sauer 1976, Martin and Stephens 1977). Grain dust consists of dirt, pieces of other plant materials, tiny fragments of grain kernels, and broken kernels. The amount of dust in grain is estimated to range from 0.01 to 1.0%. If we accept 0.05% as the average concentration of dust in grain, the total quantity of dust is 150,000 metric tons in grain in one year (Miller 1981).

The snelf life of most commercial white bread produced in the United States is only two days, even under optimum storage conditions, due to a complex phenomenon which is called bread staling. Staling results in the initial return to the bakery of an average of eight percent of the bread produced. Based on the production of 14 billion pounds per year, this represents over 1.1 billion pounds of bread per year which cannot be sold economically due to staling (Kim and D'Appolonia 1977).

OBJECTIVES

The objectives of this study were: 1) to determine the fermentable sugars released upon saccharification of grain dusts, bread waste, and cake waste; 2) to determine the yield of ethanol from hydrolyzed grain dusts, bread waste, and cake waste with and without BCS; and 3) to determine whether BCS can be used to enhance the rate of fermentation and the yield of ethanol from grain dusts, bread waste, and cake waste.

LITERATURE REVIEW

Energy from grain alcohol

1

Ethanol, or "grain alcohol", is a versatile and commercially important liquid which has been used for a variety of purposes for centuries (OTA 1981). Most industrial ethanol was produced by direct hydration of ethylene, a gas derived from petroleum or natural gas liquids. Interest in fermentation of grain and other agricultural products to produce alcohol for use as a liquid fuel has grown tremendously with the increasing cost of petroleum-derived energy sources (Klopfenstein and Abrams 1981, OTA 1981).

Historical perspective of fuel alcohol production. Ethanol fermentation can be assumed to be the first microbial process used by man; its use can be traced back some 6000 years into

Sumerian and Egyptian times. By the 14th century A.D. the distillation of alcoholic spirits from fermented grain, a practice thought to have originated in China or the Middle East, was common in many parts of the world (Demain and Solomon 1981). Until recently, however, this bioprocess has served mainly the purposed of producing beverages (Faust et al 1983).

The use of alcohol as a fuel for the internal combustion engine goes back to the invention of that engine by Dr. Nikolaus August Otto in 1861. Henry Ford believed that alconol was the best fuel for his early cars and he provided a means on the dashboard to adjust the engine for operation with either alcohol or gasoline (Scheller 1981). During World War II, largely as a war effort through government sponsorship, ethanol-gasoline mixes for automotive fuel were common in Europe (Cheremisinoff 1983). However, due to the early availability of gasoline, this technology was not utilized from the 1920's to the late 1970's. In the 1970's, with a tremendous increase in oil prices and in some areas the total non-availability of oil, alcohol once again received attention (Lyons 1983). In recent years, alconol fuel production has expanded rapidly due to federal and state incentives to encourage production from domestically abundant renewable resources. In the United States, consumption of fuel alcohol rose from about 81 million gal in 1981 to 430 million gal in 1983, and is projected to reach 850 million gal by 1990 (Gill and Allen 1985).

In Brazil, the world's leading producer of alcohol,

production has increased from 147 million gal during the 1975/76 crop year to 1.8 billion gal in 1984/85. The target for 1985/86 is 3.0 billion gal (Gill and Allen 1985, Rothman et al 1983).

Encouraged by programs within the U.S. and Brazil, many other countries began their own fuel alcohol program, and operating plants now exist in Canada, New Zealand, and the Philippines (Lyons 1983).

Fuel economy of ethanol. Ethanol may be used as a neat fuel (100% ethanol) or in gasoline blends. In the United States, most fuel ethanol is used in gasoline blends. A mixture of 10% ethanol (fermented from agriculture materials) and 90% unleaded gasoline (10/90) is called gasohol (Gill and Allen 1985, NAFC 1980a).

Ethanol has a Btu content significantly higher than that of methanol (approximately 12,780 Btu/lb vs 9,500 Btu/lb for methanol). However, ethanol's Btu value is still significantly lower than gasoline's. A gallon of ethanol contains about 70% of the Btu capacity of gasoline. The addition of ethanol to gasoline causes the Btu capacity to drop (Cheremisinoff 1983). If fuel efficiency were proportional to enthalpy of combustion, one would expect an approximate 4% decrease in miles per gallon with gasonol, compared with gasoline (Chambers et al 1979). However, in the practical use of gasohol, the lower heating value of ethanol is offset by its octane-boosting properties, high

| fuel/air ratio for combuscion in automobile engines, and greater volumetric efficiency due to the higher compression ratio, better ignition, and higher burning rate (Rothman et al 1983). A comparison between the properties of iso-octane and ethanol is presented in Table 1. Gill and Allen (1985) reported that ethanol could be used in place of tetra-ethyl lead to increase the octane rating of unleaded gasoline because of its high octane rating of 110-112.

Scheller (1974) noted at least three factors that were important to the future of gasonol, namely, the price of gasoline, the price of grain, and the value of by-products from the alcohol manufacturing process.

2 Energy savings with fuel alcohol. The energy objective of using alcohol fuels from biomass is the displacement of foreign oil and gas with domestic synthetic fuels. The effectiveness of a fuel alcohol program depends on the energy consumed in growing and harvesting the feedstock and converting it into alcohol, the type of fuel used in the conversion process, and the use of the alcohol (OTA 1981).

A number of investigators have studied energy balances with different sets of assumptions regarding variables such as energy requirements for agricultural production, energy credits allotted to by-products, conversion plant design, yield of alcohol from grain feedstock, etc. (Katzen 1979, NAFC 1986a, OTA 1981, Rothman et al 1983, Scheller and Mohr 1976).

Table 1. Comparison 3etween the Properties of Iso-octane and $\operatorname{Ethanol}^{\mathbf{a}}$

	Iso-octane (C ₈ H ₁₈)	Ethanol (C ₂ H ₅ OH)
Molecular Weight	114.224	46.07
Carbon Content, wt %	84.0	52.0
Hydrogen Content, wt %	16.0	13.0
Oxygen Content, wt %	3.9	35.0
Boiling Point, ${}^{\mathrm{O}}\mathrm{C}$ at 1 atm	99.24	78.3
Freezing Point, ^O C at 1 atm	-107.4	-114.1
Heat of Vaporization, Btu/lb at boiling point and 1 atm	116.9	361
Heat of Vaporization, 3tu/lb at 25°C and l atm	132	395
Heat of Combustion, Btu/lb at 25°C		
Higher heating value Lower heating value	20,556	12,780
Liquid fuel-gaseous H ₂ O	19,065	11,550
Stoichiometric Mixture, lb fuel/lb air	Ø.966	0.111
Autoignition Temperature, °C	417.8	362.8
Octane Number (research)	100	196

^aFrom Cheremisinoff (1983).

Cnambers et al (1979) studied that the energy balance for gasohol production was computed according to the following equation:

$$E = yc (10m - 9) - x$$

where E is the difference in nonrenewable energy consumption between gasohol production and gasoline production, y is the alcohol yield per busnel of corn, c is the nonrenewable energy cost to produce a gallon of gasoline, m is the relative volume efficiency of gasohol with respect to gasoline, and x is the total input energy to produce alcohol from a bushel of corn.

Results were shown to be strongly dependent on assumptions about the use of crop residues for fuel and the fuel economy rating of gasohol relative to that of gasoline. A small improvement in gasohol fuel economy resulted in dramatic improvements in the petroleum energy balance. They concluded that gasohol was close to the energy break-even point in terms of total nonrenewable energy, and gasohol was a unambiguous energy producer in terms of petroleum or petroleum-substitutable energy.

For 10 gal of automobile fuel, Scheller (1981) estimated that the energy saved through the use of gasohol compared to gasoline was equivalent in Btu's to 1.48 gal of crude oil or 1.63 gal of gasoline if the alcohol plant was fueled with coal.

NAFC(1980a) quantitatively evaluated net gains in premium

fuels that can be derived from the production and use of ethanol from biomass with the following basic concepts: a) efficient processes have notably reduced the energy needed to produce ethanol fuel; b) ethanol fuel used in gasohol can replace more liquid fuel than is consumed in its production; and c) using fuels such as coal or wood in producing ethanol effectively converts these abundant energy sources into premium liquid fuels.

Biomass raw materials for ethanol production

Raw materials for alcohol production can be divided into two basic categories: renewable biomass and nonrenewable fossil fuels, primarily coal. The renewable biomass materials include sugar and starch crops (and their derivatives such as food wastes) that can be converted into ethanol. Cellulosic biomass materials (plant fiber and its derivatives, such as paper and garbage) can be converted into either ethanol or methanol. Nonrenewable sources can be converted into methanol (Keim 1983, NAFC 1981). Table 2 gives the major biomass materials estimated to be available for ethanol production in the U.S. by 1990 and 2000.

The availability of biomass raw materials for alcohol fuel production depends on more than the size of a crop harvest or the height of a waste heap. Competition with other uses, production methods (and their commercial availability), transportation and collection costs, and distribution networks all will play a part

Table 2. Biomass Resource Base for Ethanol Production in the U.S. $^{\rm a}$

	Potential Ethanol Productio (Billions of Gallon		
Raw Material	1990	2000	
Grain	4.0	4.0	
Cellulose			
Wood	3.2	1.9	
Municipal Solid Waste	3.7	4.3	
Crop Wastes	1.5	1.5	
(Subtotal for Cellulose) (Wood, MSW, Crop Wastes)	(8.4)	(7.7)	
Sugar Crops	3.0	5.0	
Food Wastes	0.5	0.6	
Total	15.9	17.3	

^aFrom U.S. National Alcohol Fuels Commission (1981)

in determining how much and what kind of raw materials will be used in future alcohol fuels production (NAFC 1981).

In tropical countries, sugar crops are being used as raw materials for alconol production because they are available year-round. In 1975, Brazil's government established a national alcohol program (PROALCOOL) designed to produce fuel alcohol from mainly sugar cane (Stout et al 1978). Silva et al (1978) reported that sugar cane is a more efficient crop for ethyl alcohol production than sweet sorghum and cassava, from a net energy view point.

Grain is the primary fermentation feedstock in the U.S. for three reasons. First, it is widely available: cereal grains account for nearly 50% of the harvested acreage of all field crops in the U.S. Second, grain is a surplus commodity. Third, the technology for harvesting, handling, and processing grains for fermentation is well established (Cnung 1986).

Corn is by far the most common feedstock for ethanol production. A small amount of grain sorghum is used in the Southwest, but very little wheat is used to produce ethanol. Most of the alcohol produced from corn is now being used for fuel (Coble et al 1985, USDA 1985a).

Cellulosic materials are the most abundant renewable biomass on earth. However, their conversion to ethanol is presently not economical because saccharification of cellulose is inefficient. Cellulose is difficult to hydrolyze for two reasons. First, cellulose is insoluble in water and exists in a semicrystalline

state. Enzymatic or acid attack can occur only in amorphous regions and on the surface of crystals. Secondly, cellulose of practical value for the production of etnanol is rarely pure but coexists with lignin and hemicellulose in well defined anatomical structures. Physical barriers consisting largely of three-dimensional lignin reduce the accessibility to cellulose enzymes (Tsao 1985).

Knappert et al (1980) reported partial acid hydrolysis of cellulosic materials before cellulase hydrolysis increased glucose yields due to the acid's removal of hemicellulose, reduced degree of polymerization, and a possible change in the crystal structure of the cellulosic substrates.

Food processing wastes from cheese, fruit, and sweet corn are practical raw materials for alcohol production only in limited circumstances. Collection difficulties, the seasonal nature of the materials, and competition from other users render food processing wastes usable only in small operations or when the waste has been contaminated (NAFC 1981).

Ethanol production from grain by-products

In the cereal industry, complete utilization of resources is both a necessity and a challenge. In recent years, the recovery and modification of wastes has become increasingly important. The ultimate aim is more complete utilization of the raw material while minimizing pollution and waste (Finley 1981).

<u>Small scale ethanol production</u>. Most grain by-products are generated in relatively small amounts each time grain is processed. One logical way to utilize these by-products is to collect them in a small community base and use them as feedstocks for producing alcohol in small size production units.

NAFC (1980c) investigated the advantages of on-farm or small community based fuel alcohol production: a) Feedstocks are readily available and damaged grain can be used; b) The technology for small fermentation is theoretically available; and c) Existing gasoline-powered farm equipment can be modified to run on high-proof ethanol.

The U.S. Department of Energy reported that ethanol production from small size plants with the production capacity of from 13,300 gal to 1 million gal per year was 6% of total ethanol production in 1983, and will increase to 20% by 1985. However, many small scale production farm plants which flourished in the early stages have now disappeared due to poorly designed plants, lack of operating capital, and lack of technical know-how (Lyons 1983). On farm production, if handled properly, could contribute significant levels of fuel alcohol.

A small scale ethanol production plant has been developed by Coble et al (1981) based on the production capacity of 30 L (7.9 gal) per nour or 60 L (15.9 gal) per hour with additional fermentation tanks. The estimated cost of producing alcohol at a rate of 35,000 L per year was \$ 1.43 per L, and at 550,000 L per year was \$ 0.59 per L, with an overall plants efficiency of 77%.

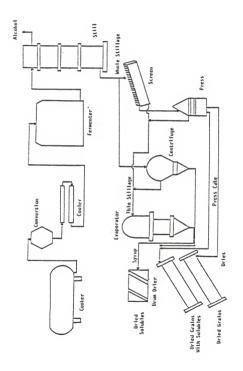
Small-scale plants with capacities of 15, 50, and 150 gal per hour were studied by NAFC (1980c) to evaluate the technical and economic feasibility of producing 190° proof and 199° proof. Notor Fuel Grade (MFG) alcohol.

Rasics of ethanol production. The production of ethanol requires four basic steps: feedstock preparation, starch conversion, fermentation, and distillation. Although not a basic step in the procedure, the collection and further processing of the fermentation by-products is usually an integral part of fuel alcohol production (Fahrennolz 1983). Fig. 1 shows a flow diagram of an ethanol production process. To maintain optimum conditions during the operation, some operational factors such as temperature and pN of the mash must be monitored carefully.

a) Feedstock preparation: Grain materials must be mechanically reduced to make the starch more accessible to the enzymes which are used in the conversion of the starch to the mono- and disaccharides required by the yeast. Grinding is the most common method of particle size reduction. While some people advocate the use of roller mills in order to reduce fines (Nellis 1979), most grains are ground through hammer mills. Particle size is important because too coarse a grind increases the time and energy required for starch gelatinization (Maisch et al 1979).

Suggested particle sizes include throughs from 1/16 in to 3/16 in screens (Caung 1986, Titus 1980). Coble et al (1981)

Figure 1. Flow diagram of an ethanol production process. (Source: Fahrenholz 1983)



used 2/16 in and 3/16 in screens for corn, but they found no significant differences in production or equipment operation.

b) Liquefaction and saccharification: Ground feedstock mixed with water is heated to gelatinize the starch and is subjected to enzymes to convert the starch to yeast-fermentable sugars. Traditionally the starch was converted to fermentable sugars with malt enzymes, prior to fermentation and distillation. recently, microbial enzymes have been used to replace malt because they provide the distiller with a reliable alternative which is easy to handle and offers considerable savings in production costs (Aschengreen 1969). Alpha-amylase hydrolyzes the alpha 1-4 bonds, forming dextrins which contain 6 to 30 glucose units (Titus 1980). Liquefaction with alpha-amylase required rather sophisticated procedures to assure dispersion of all starch molecules. A typical procedure includes adding calcium to stabilize the enzyme, adjusting the pH to 6.3-6.5, adding part of the enzyme and cooking at 105°C with steam injection and holding for 10 min. Then the mixture is heated to 140°C and held for 2 min, after which it is cooled to 90°C, and the remaining enzyme is added and the mixture held for 60 min (Keim 1983).

Saccharification is the conversion of the dextrins to the simple sugars to be utilized by the yeast. Glucoamylase (amyloglucosidase) breaks both alpha 1-4 and alpha 1-6 links to yield single glucose units (Maison et al 1979). At this stage the mash is cooled to $60-65^{\circ}\mathrm{C}$ and maintained 2 hr at pH 4.0-5.0

for complete saccharification (Keim 1983, Wu et al 1984).

c) Fermentation: Yeasts convert sugars to ethanol, carbon dioxide, and heat in the stoicniometric ratio of 2 moles each of ethanol and carbon dioxide for each mole of glucose (NRC 1981). The yeasts normally used in ethanol production are top fermenting facultative anaerobes belonging to the genus <u>Saccharomyces</u> (SERI 1980). In general, <u>S. cerevisiae</u> is especially tolerant of adverse environmental conditions, and it is generally preferred for industrial ethanol production. Mesophilic strains of <u>Saccharomyces</u> exhibit optimum cell yields and growth rates in the range of 28-35°C while the maximum temperature for growth is about 40°C (Jones et al 1981). Gray (1941) reported that one strain of <u>S. cerevisiae</u> had a lower alcohol tolerance at 35°C than at 30°C.

Hydrogen ion concentration is a significant factor in fermentation due to its importance in controlling bacterial contamination as well as its effect on yeast growth, fermentation rate, and by-product formation (Jones et al 1981). For fermentation of grain mash, initial pH was adjusted to 4.3-5.3 with either stillage equal to 23-25% of the final mash volume, or with sulfuric acid (Stark 1954). The mash for molasses, fermentation was adjusted to an initial pH of 4-5 with sulfuric acid, hydrochlolic acid, or lactic acid (Hodge and Hildebrandt 1954).

If the sugar concentration of the mash exceeds 22% by weight, the high osmotic pressure will greatly inhibit yeast

activity (SERI 1980). Fermentation will continue until the substrate is depleted or the ethanol concentration is nigh enough to destroy the yeast, greater than 12 to 14% by weight (Maisch et al 1979).

d) Distillation: The purpose of distillation is to separate the ethanol from the fermented mash. Conventional distillation procedures use a system of two columns: a stripping column to separate ethanol from the mash and a rectifying column to concentrate the ethanol. Sieve trays in the columns improve liquid-vapor contact and encourage refluxing (SERI 1980).

The formation for an azeotropic mixture of water and alcohol at 1 atm limits the concentration to 95.6% by weight of ethanol (NAFPA 1979). Anhydrous alcohol can be obtained by azeocropic distillation using benzene (NRC 1981).

MATERIALS AND METHODS

Materials

The types of grain dusts tested were wheat-corn, cornsorghum, and sorghum-soybean (2 samples each). The six 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. Bread waste was obtained from the Baking Science Laboratory of the Department of Grain Science and Industry, Kansas State University. Cake waste was obtained from the American Institute of Baking, Mannattan, KS. BCS was obtained from Anheuser-Busch, Inc. in 1985; a sample with 48.5% solids was from the brewery in Columbus, OH.

Distillers' active-dry yeast was obtained from Biocon (U.S.)
Inc., Lexington, KY. The recommended usage rate was 2-4 lbs/1300 gal (5-13 million cells/ml) of mash when the sugar concentration was between 15-25%. The optimum pH was between 4.0 and 5.5. The optimum temperature was 86° F (30°C). However, good yields were obtained between 80°F and 100°F with the rate of fermentation increasing with increasing temperature. A bacterial alphaamylase (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) was also obtained from Miles Laboratories, Inc. One m1 of Diazyme L-200 has a leveled activity of 200 Diazyme Units (DU). One DU is the amount of enzyme that catalyzes the production of 1 g of glucose from starch in 1 hr at 60°C and pH 4.2.

Methods

Sun-dried bread waste and cake waste were ground in a Burrows hammer mill using a 1/16 in (1.6 mm) screen and placed in cold storage with other grain dust samples. The moistures of grain dusts, bread waste, and cake waste were determined by evaporation at 95°C for 4 hr under vacuum of 4000 pa or 30 torr (A.O.A.C. 1984, Method 7.003). Total starch contents of the grain dusts were determined by A.A.C.C. method 76-11 (1976), and those of bread waste and cake waste were also determined using 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 (1984). Amino nitrogen was determined by A.A.C.C. method 46-31 (1976). Glucose, fructose, and ethanol were determined by highperformance liquid chromatography (HPLC) using a Varian Model 5,000 LC (Varian Associates, Inc., Palo Alto, CA) chromatograph equipped with a loop-injection device (10 ul) and a refrectometor as the detector. All separations were done using a Bio-Rad

Aminex Ion-Exclusion Column (HPX-87H, 300 mm x 7.8 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 0.9 ml/min. Sucrose was also determined by HPLC using a Beckman 100A system with Altex Model 156 refractive index detector. Sucrose was separated on an Amino Sepheri-5 column (Brownlee Labs, Santa Clara, CA). Standard curves were obtained from solutions of known concentrations of sugars and ethanol.

Liquefaction and saccharification

One hundred twenty grams (dry basis (db)) each of grain dusts, ground bread waste, and cake waste were dispersed in about 460 ml water. The pH of each slurry was adjusted to 6.2 with 2 M NaOH and 0.3 ml of TAKA-THERM was added. The temperature was maintained at 90° C for 1 hr with constant stirring to gelatinize and degrade starch to soluble dextrins. The thinned 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 hr with stirring.

For a 1:1 mixture of each sample with BCS, 300 g of slurries containing 60 g (dry solids (ds)) samples were liquefied with 0.15 ml of TAKA-THERM under the conditions described previously. Then, each thinned slurry was mixed with 300 g of 20% (w/w) BCS, and saccharified with 0.9 ml Diazyme L-200 under the same conditions described previously. BCS (20% w/w) alone was also saccharified using Diazyme L-200 in the same manner. Hydrolyzed

slurries of each sample were diluted to 15% solids (as solids content before hydrolysis) for fermentation, and if necessary, pH was readjusted to 4.2 for grain dusts, and 4.6 for bread waste and cake waste.

Fermentation

One gram yeast was rehydrated in 25 ml 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 (wet basis (wo)). The pH was readjusted to 4.2 for grain dusts and 4.6 for bread waste and cake waste.

To determine the yields of ethanol, 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 for 10 min in a Beckman Model J2-21 Centrifuge. Residual sugars and ethanol were determined using HPLC.

To determine the rate of fermentation and the optimum fermentation times, carbon dioxide gas production was followed using a 12-channel recording gasograph (Rubenthaler et al 1933). A gasograph Model 12 manufactured by D&S Instrument Lcd. (Pullman, WA) was used in this experiment. A test tube (15 ml) containing 7 g substrate 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 cnannel, which had a recording pen. Optimum fermentation times

found by the gasograph were 10-15 hr shorter than those found by ethanol production.

RESULTS AND DISCUSSION

Proximate Analysis

All samples were analyzed for total starch, crude protein, crude fat, and crude ash. Data are presented in Table 3. Grain dusts contained high amounts of ash and varied widely in starch content even though the sources of grain, from which the dusts came, were the same. Average sugar compositions in enzymedigested bread waste and cake waste were measured by HPLC during preliminary work and values are presented in Table 4. Sucrose in the bread waste might have come from non-yeast bread or from sucrose-containing ingredients added after baking.

Fermentation of Hydrolyzed Grain By-products and Their Mixtures with BCS

From the results shown in Table 3, grain dust samples chosen for fermentation were low and high starch wheat-corn dust, low and high starch corn-sorghum dust, and a 1:1 mixture of the two sorghum-soybean dust samples.

The rates of fermentation were measured by carbon dioxide production during fermentation using the gasograph. The gasograph

Table 3. Proximate Chemical Composition of Grain By-products and BCS (dry basis)^a

Source	Total Starch (%)	Crude Proteinb (%, N x 6.25)	Crude Fat (%)	Crude Ash (%)		
Wheat-corn						
Dust 1	27.0±0.8	10.4 <u>+</u> 1.1	4.2 <u>+</u> 0.6	20.9±0.1		
Dust 2	41.9 <u>±</u> 0.5	8.9 <u>+</u> 0.6	3.9 <u>+</u> 0.3	17.7±1.3		
Corn-sorghum						
Dust 1	29.5 <u>+</u> 1.0	10.3 <u>+</u> 0.7	4.1±0.4	19.1±0.3		
Dust 2	41.7 <u>±</u> 0.5	9.1 <u>+</u> 0.5	4.2 <u>+</u> 0.5	15.2 <u>±</u> 0.4		
Sorghum-soybe	ean					
Dust 1	37.9 <u>±</u> 0.6	8.3 <u>+</u> 0.7	3.3±0.1	20.3±0.7		
Dust 2	40.8 <u>+</u> 0.6	9.6 <u>+</u> 1.0	3.6 <u>+</u> 0.3	15.4 <u>+</u> 0.4		
Bread waste	66.2 <u>±</u> 0.5	12.1 <u>±</u> 0.9	2.2 <u>+</u> 0.1	2.0±0.1		
Cake waste	32.4±0.2	5.7 <u>+</u> 0.3	16.9 <u>+</u> 0.1	2.1 <u>±</u> 0.1		
BCS ^C	-	8.9 <u>+</u> 1.1	1.4±0.3	2.5±0.2		

 $^{^{}m a}$ Each value is a mean of four replications \pm S.E. except BCS.

 $^{^{\}mbox{\scriptsize b}}\mbox{\scriptsize The nitrogen factor of 5.7}$ was used for bread waste and cake waste.

CFrom Sebree et al (1983).

Table 4. Average Sugar Composition in Saccharified Bread Waste and Cake Waste

Sugars	Bread Waste (g/100g ds)	Cake Waste (g/100g ds)
Glucose	67.1	34.2
Sucrose	1.3	28.3
Fructose	0.3	2.2

was introduced by Rubenthaler et al (1980) 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 may also be expressed in cc by multiplying GU by 2.38.

The results of fermenting hydrolyzed grain dusts and the mixture of BCS and each sample (1:1) are given in Table 5, and also are plotted in Figures 2 to 4. Optimum fermentation times measured by gasograph are reported in Table 6. For grain dusts containing low starch, gas production leveled off in 20 hr, and high starch in 25-26 hr. For 1:1 mixtures of BCS and grain dusts, the gas production reached its peak in 25-26 hr. When equal amounts of BCS were added to grain dusts, optimum fermentation times were not reduced, but carbon dioxide produced during fermentation was markedly increased, probably due to the high amounts of available carbon in BCS (Table 8). Table 7 and Fig. 5 show the results of gas production from bakery wastes, and from the mixture of BCS and each sample (1:1). Addition of BCS to bread waste and cake waste did not increase CO2 gas production but did reduce the optimum fermentation times from 62 hr to 34 hr, and from 76 hr to 35 hr, respectively. This significant reduction (p<0.05) of optimum fermentation time might be due to some nutrients in BCS. Chung (1986) reported that addition of BCS to corn, grain sorghum, and wheat resulted in the

Table 5. Carbon Dioxide Production during Fermentation of Saccharified Grain Dusts and Their Mixtures with 3CS

		co ₂	Produc	tion (GU~)		
Source	8	12	16	20	24	28	32(nr)
Wheat-corn dust							
Low-starch (WLS)	4.4	11.1	21.3	30.1	29.7	28.9	-
WLS + BCS (1:1)	5.0	12.6	25.2	43.3	52.2	52.1	51.2
High-starch (WHS)	5.6	14.4	24.8	36.5	42.9	44.4	44.1
WHS + BCS (1:1)	7.0	18.3	32.6	48.3	57.2	53.3	57.5
Corn-sorghum dust							
Low-starch (CLS)	6.2	16.2	27.9	31.8	31.4	31.1	-
CLS + 3CS (1:1)	5.3	13.3	25.8	42.6	52.2	52.1	51.2
High-starch (CHS)	6.2	16.4	29.4	40.6	44.1	44.3	43.4
CHS + BCS (1:1)	4.6	11.4	22.2	38.8	56.1	58.5	57.4
Sorghum-soybean dust (SSD)	4.5	11.3	22.6	34.4	40.6	40.7	40.3
SSD + BCS (1:1)	3.9	9.8	19.7	35.8	51.0	55.2	54.6

 $^{^{}a}$ GU X 2.38 = cc, or GU X 7.3 = mm Hg.

Figure 2. Carbon dioxide production during fermentation of saccharified mixtures of wheat-corn dust and BCS. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.2. GU X 2.38 = cc, or GU X 7.3 = mm Hg.

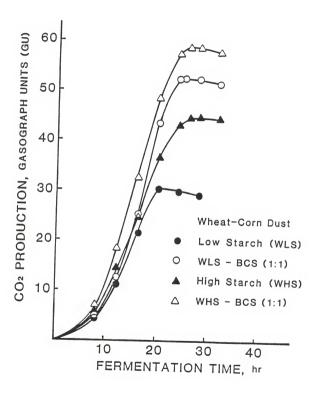


Figure 3. Carbon dioxide production during fermentation of saccharified mixtures of corn-sorghum dust and 3CS. Fermentations were done using 15% solids with U.2 g dry yeast/kg at 30°C and pH 4.2. GU x 2.38 = cc, or GU x 7.3 = mm Hg.

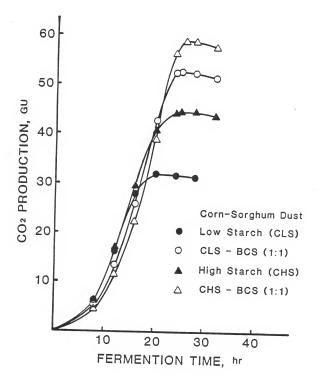


Figure 4. Carbon dioxide production during fermentation of saccharified mixtures of sorghum-soybean dust and BCS. Fermentations were done using 15% solids with 3.2 g dry yeast/kg at 33°C and pH 4.2. GU X 2.38 = cc, or GU X 7.3 = mm Hg.

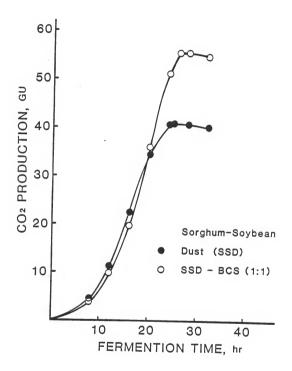


Table 6. Ethanol Yields Produced by Yeast Fermentation of Saccharified Grain By-products and Their Mixtures with Glucoamylase-Treated BCS; Optimum Fermentation Times and Fermentation Efficiencies.

	Total Fermentabl	e	Ethanol Yield ^b	l 	Optimum Fermentation Time ^C	Fermentation Efficiency d
Source	(% db)	(m1/)	(g ds)	(gal/ton)	(nr)	(%)
Wneat-corn dust						
Low-starch (WLS)	28.0	164ª	± 7.5	39	20ª	90.4
WLS+BCS (1:1)		3Ø5 ^b	± 4.5	73	25 ^b	
High-starch(WHS	43.3	263 ^a	± 3.8	63	26ª	93.7
WHS+BCS (1:1)		353b	± 5.6	85	26 ^a	
Corn-sorghum dust						
Low-starch (CLS)	30.6	177ª	± 5.9	42	20ª	39.4
CLS+BCS (1:1)		308b	± 7.0	74	25 ^b	
Hign-starch (CHS	43.9	262 ^a	± 3.3	63	25 ^a	92.1
CHS+BCS (1:1)		347 ^b	± 6.5	83	26ª	
Sorghum-soybean dust(SSD)	40.5	229ª	± 6.7	55	25ª	87.3
SSD+BCS (1:1)		343 ^b	± 6.8	82	26ª	
Bread waste(BW)	63.8	427ª	± 5.0	102	62 ^a	95.7
BW+BCS (1:1)		441 ^a	±11.6	106	34 ^b	
Cake waste(CW)	66.2	4J5ª	± 9.1	97	76ª	94.4
CW+BCS (1:1)		437 ^b	±10.7	105	35 ^b	

^aTotal fermentable sugars were determined after hydrolysis. Total fermentable sugars included glucose, sucrose, and fructose. All sucrose were assumed to be converted to glucose and fructose

 $^{^{\}rm b}$ Each value is the mean of 4 replications \pm S.E. Mean comparisons within every two rows followed by the same letter are not significantly different (p(0.35).

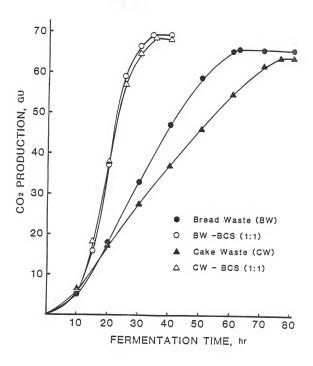
General value is the mean of 2 replications. Mean comparisons within every two rows followed by the same letter are not significantly different (p(3,05).

Table 7. Carbon Dioxide Production during Fermentation of Saccharified 3akery Wastes and Their Mixtures with BCS $\,$

	CO ₂ Production (GU ²)								
Source	10	20	30	35	43	50	6 ป	70	80(nr)
Bread waste (BW)	5.2	17.8	33.0	39.5	47.0	58.6	65.1	65.2	64.9
BW + BCS (1:1)	6.1	37.3	66.3	69.1	68.9	-	-	~	-
Cake waste (CW)	5.9	17.3	27.5	31.6	37.0	46.0	54.5	61.3	63.3
CW + BCS (1:1)	6.6	37.8	64.5	68.2	67.9	-	-	-	-

 $^{^{}a}$ GU X 2.38 = cc, or GU X 7.3 = mm Hg.

Figure 5. Carbon dioxide production during fermentation of saccharified mixtures of bakery wastes and BCS. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.6. GU X 2.38 = cc, or GU X 7.3 = mm Hg.



reduction of fermentation time (Table 8), and maximum fermentation benefit could be obtained when the mixture ratio was 1:1.

The fermentation rate of cake waste was also followed by measuring the ethanol content in ferments done in water-sealed flasks. Fig.6 and Table 9 represent the sugar consumption and ethanol production during fermentation of cake waste. Sucrose was quickly hydrolyzed to glucose and fructose by yeast invertase in the first 10 hr of fermentation, and fructose was consumed by yeast at a significantly slower rate than glucose. These data are in agreement with what Kulp et al (1985) observed in fermentation of liquid ferments for white pan bread. Similar observations were also reported for straight doughs by Koch et al (1954).

Fermentation rate measured by ethanol production showed a trend similar to that found by the gasograph (Fig. 5). Ethanol production was almost complete in 90 hr. However, CO_2 production appeared to be completed 14 hr earlier. This phenomenon might be due to CO_2 absorption in the water, and reduction of total CO_2 volume by the increased pressure in the gasograph jar. The declines in total CO_2 after the peaks support this explanation. Also, there was a decrease in the pH of the water (5.7 to 3.9-4.0) in the jar after fermentation, indicating probable CO_2 absorption by the water.

The effects of pH on fermentation of hydrolyzed bread waste and cake waste are presented in Table 10. The large drop in pH

Table 8. Ethanol Yields and Optimum Fermentation Times Obtained from Yeast Fermentations of Enzyme-Digests of Grains Mixed with Glucoamylase-Treated BCS^a

Source	Glucose Released by Enzyme Hydrolysis (% db)	Ethanol Yield (ml/kg ds)	Optimum Fermentation Time (hr)
Corn	73.7	429	61
Corn+BCS(1:1)		443	33
Sorghum	75.7	448	65
Sorghum+BCS(1	:1)	448	33
Wheat	69.0	403	57
Wheat+BCS(1:1	.)	440	33
BCS	76.4	460	29

aFrom Chung (1986).

Table 9. Sugar Consumption and Ethanol Production during Fermentation of Cake Waste

Fermentat Time Et			Sugar (g/100g ds)	
(hr)	(ml/100g ds)	Glucose	Fructose	Sucrose
Ø	Ø	34.2	2.2	28.3
10	2.7	44.0	16.4	1.4
20	8.3	35.2	16.3	1.4
30	14.1	27.1	15.1	1.4
40	19.9	19.1	13.6	1.4
50	24.9	12.6	12.1	1.4
60	28.4	8.1	10.2	1.4
70	32.0	4.3	8.7	1.4
80	37.6	1.7	4.5	1.4
90	40.0	0.9	1.6	1.4
100	40.5	Ø.9	1.1	1.4

41

Figure 6. Sugar consumption and ethanol production during fermentation of cake waste. Fermentations were done using 15% solids with 0.2 g dry yeast/kg at 30°C and pH 4.6.

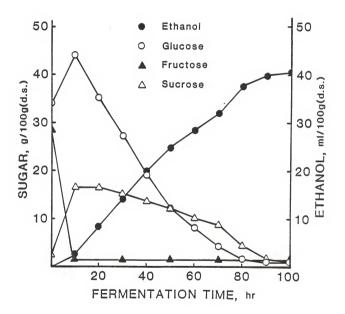


Table 10. Effect of pH on Fermentation of Saccharified Bread Waste and Cake Waste.

Source	Initial pH	Final pH	Fermentation Time (hr)	CO ₂ Production at Peaks ^a (GU ^b)
Bread waste	4.2	3.6	63	66.2 ^a
	4.6	3.8	62	65.5 ^a
	5.0	4.0	65	65.5 ^a
Cake waste	4.2	3.4	78	62.7ª
	4.6	3.5	76	63.3 ^a
	5.0	3.6	77	62.5 ^a

 $^{^{\}rm a}\rm Each$ value is the mean of 2 replications. Mean comparisons within every three rows followed by the same letter are not significantly different (p<0.05).

 $^{^{}b}GU \times 2.38 = cc$, or $GU \times 7.3 = mm$ Hg.

after fermentation implied that bread waste and cake waste had poor buffer capacities.

Fermentation of sucrose and fructose solutions is more sensitive to pH than fermentation of glucose. The control of brew pH affects the sugar utilization, allowing a nigh fermentation rate to be maintained (Jones et al 1981, Kulp et al 1985). Because cake waste contained relatively large amount of sucrose and fructose (about 30% db), optimum pH during fermentation was expected to give a high rate of fermentation. Carbon dioxide production during fermentation of bread waste and cake waste was not affected (p < 0.05) by the initial pH, which ranged from 4.2 to 5.0. However fermentation times were slightly reduced when the initial pH was adjusted to 4.6.

Yields of Ethanol

Total fermentable sugars in grain by-products after successive treatments with alpha-amylase and glucoamylase were measured by HPLC. Glucose, sucrose, and fructose were included in total fermentable sugars and the values, which were proportional to the starch content except for cake waste, are presented in Table 6. Chung (1986) found that maltose was not quantitated in the digests because it eluted together with isomaltose. Coble et al (1981) reported that the average efficiency for conversion of starch to sugar was 90% when corn and grain sorghum were hydrolyzed using alpha-amylase and

glucoamylase in a small scale ethanol production plant.

The yields of ethanol from hydrolyzed grain by-products and their mixtures with hydrolyzed BCS are also reported in Table 6. The ethanol production by fermentation of grain dusts ranged from 164 ml/kg ds to 263 ml/kg ds. Addition of BCS in grain dusts increased ethanol production by 32-86%, with yields of 305 ml/kg ds to 353 ml/kg ds When BCS was added in bakery wastes, no significant difference (p<0.05) in ethanol yield was found in bread waste, but ethanol yield was increased about 7% in cake waste. In all cases, only trace amounts of residual sugars were detected after fermentation.

Adding BCS did not increase etnanol yield in corn and sorghum fermentation (Table 8), because BCS and these grain materials gave about equal amounts of glucose upon enzyme hydrolysis. But the yield was slightly increased in wheat fermentation (Chung 1986).

The fermentation efficiencies of grain by-products were generally more than 90% and tended to get higher as total fermentable sugar amounts increased in hydrolyzed substrates (Table 6). Bakery wastes were very high in fermentation efficiencies (94-96%) and sorghum-soybean grain dust was the lowest (87%). Stark (1954) reported that fermentation efficiency was an index of the physiological condition of the yeast, and that overall processing plant efficiency was a standard for the evaluation of all process operations, from handling of the raw materials through fermentation, or through distillation if based

on the alcohol in storage tank. He found that 92-95% fermentation efficiencies (plant basis) were obtained for corn, 82-90% for wheat, and 93% for grain sorgnum. Coble et al (1981) reported that the average efficiency for fermentation of sugar to alcohol was 90% with overall plant efficiency of 77% when corn and grain sorghum were used as feedstocks for small scale ethanol plant.

Amino Nitrogen in Hydrolyzed Grain By-products and BCS

The nitrogen content of yeasts is about 10% of the dry weight, representing that nitrogen is an important constituent of any growth medium (Jones et al 1981). The amino nitrogen content in hydrolyzed grain by-products and BCS was determined as formal nitrogen by the method of Sorenson, and the values are presented in Table 11. With the amounts of 67-81 mg/100g ds, grain dusts were relatively rich in amino nitrogen compared to grain itself (Table 12). Bread waste and cake waste contained very low concentrations of amino nitrogen, with amounts of 15 mg/100g ds and 12 mg/100g ds, respectively. BCS had 3-20 times more amino nitrogen than any of the grain by-products.

Chung (1986) 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 nitrogen to the hydrolyzed grains markedly increased the rate of fermentation.

Table 11. Amino Nitrogen in Saccharified Grain By-products and BCS $\,$

Source	Amino Nitrogen ^a (mg/l00 g ds)
Wheat-corn dust	
Low-scarch	78.5 ± 1.3
High-starch	67.4 ± 1.0
Corn-sorghum dust	
Low-scarch	80.9 ± 0.7
High-starch	75.8 \pm 1.3
Sorghum-soybean dust	72.1 ± 2.9
Bread waste	14.8 ± 0.1
Cake waste	11.7 ± 0.7
BCSb	230.0

 $^{\rm a}$ Each value is a mean of two samples \pm S.E.

bFrom Chung (1986)

Table 12. Amino Nitrogen in Hydrolyzed Grain Materials and Their Optimum Fermentation times $^{\rm a}$

Source	Amino Nitrogen (mg/100 g ds)	Optimum Fermentation Time (hr)
Corn	39	61
Sorghum	33	65
Wheat	31	57
Wheat screenings	81	25
Low-grade flour	72	31

aFrom Chung (1986).

Kirsop and Brown (1972) have shown that if the concentration of all the noncarbohydrate constituents of malt wort were halved, the rate of fermentation was reduced but it could be completely restored by the addition of serine or arginine. They also found that the rate of fermentation was proportional to the values for amino nitrogen (Table 13), suggesting that exhaustion of nitrogenous compounds was the limiting factor for yeast growth.

Addition of BCS to hydrolyzed bakery wastes significantly reduced the fermentation times (Table 6). This effect might be due to the high amino nitrogen content of BCS. With equal amounts of initial glucose, adding BCS to grain dusts did not reduced (p<0.05) fermentation times (Table 14). This phenomenon suggested that grain dusts had enough nitrogen content for yeast growth. From the results shown in Tables 6, 11, and 12, a good linear relationship (r = -0.97) was found between optimum fermentation time and amino nitrogen in the hydrolyzed grains and their by-products (Fig. 7).

Cost Analysis for Ethanol Production

Grain dust collected from elevators is frequently returned to the grain and moves with the grain through the marketing channels (Martin and Sauer 1976). In some grain elevators in northeast Kansas, about 3-5% of the grain dust is returned to the grain, and the rest of it is discarded or given away for animal feed. Stale bread and cake is sometimes collected by a

Table 13. Time Required for Fermentation and Amino Nitrogen Content of Jarious Worts $^{\rm a}$.

Wort		Growth	Fermentation Time (hr)
Α	9.5	1.3	79
В	14.5	1.9	56
С	16.0	2.4	56
D	19.0	2.6	43
E	23.0	2.8	38
F	24.0	2.9	35
G	28.0	3.2	29
H	34.0	3.3	28

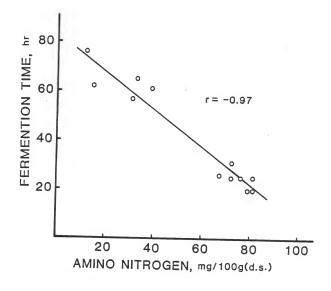
aFrom Kirsop and Brown (1972).

Table 14. Fermentation Times of Saccharified Grain
Dusts and Their Mixtures with BCS Based
on Equal Initial Glucose Contents
(5.6% wb)

Source	Optimum Fermentation Time ^a (hr)
Wheat-corn dust	
Low-starch (WLS)	22 ^a
WLS + BCS (1:1)	23 ^a
	25a
Hign-starch (WHS)	254
WHS + BCS	25 ^a
Corn-sorgnum dust	
Low-starch (CLS)	22 ^a
CLS + BCS (1:1)	24 ^a
High-starch (CHS)	23 ^a
CHS + BCS (1:1)	24 ^a
Sorghum-soybean dust (SSD)	23a
dust (SSD)	43-
SSD + BCS (1:1)	25 ^a

^aEach value is a mean of 2 replications. Mean comparisons within every two rows followed by the same letter are not significantly different (p<0.35).

Figure 7. Relationship between amino nitrogen in enzyme hydrolyzed substrates and their fermentation times measured by gasograph.



distributor and sold for animal feed at \$1 per bucket (50-100 lbs), but this bakery waste is usually discarded.

Estimated net feedstock costs for grain dust, bakery waste, and grains, to produce one gallon of absolute ethanol, with and without added BCS, are presented in Table 15. Since the cost of plant-derived feedstock represents over 50% of the total cost for ethanol production (Faust et al 1983), it is important to identify economical feedstocks. The total cost of the grain dust was calculated as 5% of the corn price (\$2.34/bu * 0.05 = \$0.12/bu), because about 5% of the dust was mixed back with the grain (mostly corn), and the other grain dust was free. The cost of bakery waste was assumed to be \$1/100lbs. BCS is presently selling around \$20/ton. Feedstock costs for grain dust and bakery waste might be decreased when these feedstocks are purchased on a regular basis.

Based on a typical small scale ethanol production plant (50 gal of ethanol per hr), total costs for producing one gallon of ethanol from grain dust, bakery waste, and grains, with and without BCS, were calculated and are shown in Tables 16 and 17. The U.S. National Alcohol Fuels Commission (1980b) has performed a detailed cost calculation on a 300,000 gallon per year (50 gal per hr) for producing 190° proof fuel ethanol from corn (Table 18). Twenty five percent inflation from 1981 dollars was applied for calculating fixed and variable cost. By-product credit was not subtracted from the estimated production cost, but it could be an important factor.

Table 15. Estimated Feedstock Costs from Various Raw Materials and Their Mixtures with BCS

Raw Material ^a	Price	Ethanol Yield	Net Feedstock Cost
Grain dust (GD)	12.40	52	0.24
GD + BCS ^b (1:1)	26.20	79	0.33
Bakery waste (BW)	32.25	100	0.32
BW + BCS (1:1)	36.13	106	0.34
Corn	94.94	103	Ø.92
Corn + BCS (1:1)	67.47	106	0.64
Sorghum	87.23	107	0.82
Sorghum + BCS (1:1)	63.62	107	0.59
Wheat	124.20	97	1.28
Wheat + BCS (1:1)	82.10	105	Ø.78

^aBased on cash price of grain at Kansas City Market on May 19, 1986: corn (No.3), \$2.34/bu; sorghum (No.3), \$2.15/bu; and wheat (No.3), \$3.28/bu. Moisture content for grain and grain dust = 12%, and bakery waste = 38% Bulk density of grain dust = 22 lbs/bu, corn and sorgnum = 56 lbs/bu, and wheat = 60 lbs/bu.

^bThe price of BCS was assumed to be \$20/ton. Solid content of BCS = 50%.

Table 16. Costs for Ethanol Production from Various Feedstocks and Their Mixtures with BCS^a

Feedstock	/1h 3-/h-1	Ratio of Fermentation Time ^C	Costa
Grain dust (GD)	2140	0.38	0.37
GD + BCS (1:1)	1410	0.42	0.27
Bakery waste (BW)	1120	1.13	0.57
BW + BCS (1:1)	1050	0.57	0.27
Corn	1080	1.00	0.48
Corn + BCS (1:1)	1050	0.54	0.26
Sorghum	1040	1.07	0.50
Sorghum + BCS (1:1)	1040	0.54	0.25
Wheat	1150	0.94	0.49
Wheat + BCS (1:1)	1060	0.54	0.26

 $^{^{\}rm a}{\rm Based}$ on 190 $^{\rm o}$ proof alcohol from corn (NAFC 1980b). Alcohol production rate = 50 GPH.

bDistillation efficiency = 95%. Alcohol recovery = 95%.

^CRelative to the fermentation time of corn (61 hr).

 $^{^{\}rm d}{\rm Variable}$ cost was assumed to be proportional to the feed rate and the ratio of fermentation time.

Table 17. Total Production Cost for Ethanol from Various Feedstocks and Their Mixtures with BCS^a (S/gal ethanol)

Feedstock	Cost	Costb	Cost ^C	Total Production Cost
Grain dust (GD)	0.24	0.54	Ø.37	1.15
GD + BCS (1:1)	0.33	0.54	Ø.27	1.14
Bakery waste (BW)	0.32	0.54	Ø.57	1.43
BW + BCS (1:1)	0.34	0.54	0.27	1.15
Corn	0.92	0.54	0.48	1.94
Corn + BCS (1:1)	0.64	0.54	Ø.26	1.44
Sorghum	0.82	0.54	0.50	1.86
Sorghum +BCS (1:1)	0.59	0.54	Ø.25	1.38
3			5.25	2.55
Wheat	1.28	0.54	0.49	2.31
Wheat + BCS (1:1)	0.78	0.54	0.26	1.58

 $^{^{\}rm a}{\rm Based}$ on 190° proof fuel alcohol from corn (NAFC 1980b). Alcohol production rate = 50 gal per hr (GPH).

 $^{^{\}mbox{\scriptsize b}}\mbox{Fixed cost}$ included depreciation (10 yr), maintenance, and insurance.

 $^{^{}C\!V}$ ariable cost included electricity, fuel(coal), labor, enzyme, yeast, and other chemicals.

dBy-product credit was not included.

Table 18a

Total Production Cost - 1981 Basis 1900 Proof Fuel Alcohol from Corn 50 GPH - Base Case 300,000 GPY Production TFI = \$714,000

	\$/yr	\$/gal
Fixed charges		
Depreciation (10 yr), 10% TFI Maintenance, 6% TFI Taxes and insurance, 2% TFI	71,400 42,840 14,260	0.238 0.143 0.047
	128,500	0.428
Raw materials Corn (\$2.70/bu) Enzyme (\$0.88/lb, liquid) Yeast (\$1.00/lb, cake) Other chemicals	356,400 21,000 4,000 1,500	1.188 0.070 0.013 0.005
	382,900	1.276
Utilities Electric power (\$0.035/kwh) Fuel (Coal, \$40/T)	15,130 27,000	0.050 0.090
	42,100	3.149
Labor 1 Operator * 3 Shifts * \$15,000/yr	45,000	Ø.150
Total production cost	598,500	1.994

TFI = Total Fixed Investment

aFrom NAFC (1980b)

Dehydration of stillage from small-scale production systems does not appear promising because of the high energy requirements and costs (Coble et al 1981). However, the whole stillage could be sold for animal feed as Wet Distiller's Grains (WDG) containing 25-35% dry solids by using low energy methods such as screening, pressing, and sedimentation.

Compared to using grain as a feedstock, ethanol production from grain dust cost only half as much, and ethanol from bakery waste cost three-fourths as much (Table 17). Adding BCS to grains decreased the ethanol production cost by 25-30%. The cost was also decreased by 20% when BCS was added to bakery waste. The cost was only slightly decreased when BCS was added to grain dust. This result might be due to the relatively high price of BCS compared to that of grain dust. However, more cost benefit would be observed if the cost estimation had included the higher productivity and better by-product credit resulting from adding BCS.

CONCLUSIONS

Grain by-products such as grain dusts and bakery wastes could be used as good feedstocks for ethanol production with high fermentation efficiencies. When BCS was added to these hydrolyzed substrates, fermentation was improved in two ways: one for the fermentation time and the other for the ethanol yield.

In some grain by-products which were low in amino nitrogen, exhaustion of nitrogenous compounds in substrates was determined to be a limiting factor for yeast growth. Because BCS was a very rich source of nitrogen, adding BCS to saccharified bread waste and cake waste reduced fermentation time from 62 hr to 34 hr and from 76 hr to 35 hr, respectively. Addition of BCS in grain dusts did not reduce the fermentation time due to the high concentration of assimilable nitrogen in grain dusts themselves. When hydrolyzed grain by-products were low in fermentable sugar content, addition of BCS increased the yields of ethanol because of the high content available carbon in BCS. The yields of ethanol were increased from 164 - 263 ml/kg ds to 305 - 353 ml/kg ds on grain dusts when equal amounts of BCS were added. However, adding BCS only slightly increased the ethanol yields from bakery wastes.

Compared to using grain as a feedstock, ethanol from grain dust cost only half as much, and ethanol from bakery waste cost three-fourths as much. Adding BCS to grains decreased the ethanol production cost by 25-30%. The cost was also decreased by 20% when BCS was added to bakery waste. The cost was only slightly decreased when BCS was added to grain dust. This result might be due to the relatively high price of BCS compared to that of grain dust. However, more cost benefit would be observed if the cost estimation had included the higher productivity and better by-product credit resulting from adding BCS.

LITERATURE CITED

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved Methods of the AACC. The Association, St. Paul, MN.
- ASCHENGREEN, N. H. 1969. Microbial enzymes for alcohol production. Process Biochem. 8: 23.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1984. Official Methods of Analysis, 14th ed. The Association, Washington, D.C.
- CHAMBERS, R. S., HERENDEEN, R. A., JOYCE, J. J., and PENNER, P. S. 1979. Gasohol: Does it or doesn't it produce positive net energy? Science 206: 789.
- CHEREMISINOFF, N. P. 1983. Gasohol for energy production. Energy Technology Series. Ann Arbor Science Publishers Inc., Ann Arbor, Mich.
- CHUNG, K. M. 1986. Viscosity Reduction of Brewers' Condensed Solubles (BCS) and Their Use on Ethanol Production from Grain Materials. Ph.D. Dissertation. Kansas State University, Manhattan.
- COBLE, C. G., HILER, E. A., SWEETEN, J. M., O'NEAL, H. P., REIDENBACH, V. C., LE POLI, W. A., ALDRED, W. H., SCHELLING, G. T., and RAY, R. D. 1981. Small-scale ethanol production from cereal feedstocks. p 611 in: Cereals. A Renewable Resource. Theory and Practice. Y. Pomeranz and L. Munck, eds. The American Assoc. of Cereal Chemists, Inc., St. Paul, NN.
- COBLE, C. G., SWEETEN, J. M., EGG, R. P., SOLTES, E.J., ALDRED, W. H., and GIVENS, D. A. 1985. Biological conversion and fuel utilization: Fermentation for ethanol production. p 113 in: Biomass Energy. A Monograph. E. A. Hiler and B. A. Stout, eds. Texas A&M University Press, College Station, TX.
- DEMAIN, A. L., and SOLOMON, N. A. 1981. Industrial microbiology. Scientific American 245(3): 67.
- ENVIRNMENTAL PROTECTION AGENCY. 1985. Regulation of fuels and fuel additives: Gasoline lead content. Federal Register. 50: 9386.
- FAHRENHOLZ, C. H. 1983. A Study on the Quality of Whole Stillage When Damaged Grains are used as Feedstocks for Alcohol Production. MS Thesis. Kansas State University, Mannattan.

- FAUST, U., PRAVE, P., and SCHLINGMANN, M. 1983. An integral approach to power alcohol. Process Biochem. 18(3): 31.
- FINLEY, J. W. 1981. Utilization of cereal by-products. p 545 in: Cereals. A Renewable Resource. Theory and Practice. Y. Pomeranz and L Munck, eds. The American Assoc. of Cereal Chemists, Inc., St. Paul, MN.
- GILL, M. and ALLEN, E. 1985. Status of the U.S. ethanol market. p 14 in: Feed, Outlook and Situation Report, FDS-297. Economic Research Service, United States Department of Agriculture.
- GRAY, W. D. 1941. Studies on the alcohol tolerance of yeasts. J. Bact. 42: 561.
- HODGE, H. M., and HILDEBRANDT, F. M. 1954. Chap. 3. Alcohol fermentation of molasses. p 73 in: Industrial Fermentation, Vol I. L. A. Underkofler and R. J. Hickey, eds. Chemical Publishing Co., Inc., New York, NY.
- JONES, R. P., PAMMENT, N., and GREENFIELD, P. F. 1981. Alcohol fermentation by yeasts - the effect of environmental and other variables. Process Biochem. 16: 42.
- KATZEN, R. ASSOC. 1979. Grain motor fuel alcohol, Technical and economic assessment study. U.S. Dept. of Energy. Contract No. EJ-78-C-91-6639. Washington, D.C.
- KEIM, C. R. 1983. Technology and economics of fermentation alcohol - an update. Enzyme Microbiol. Tech. 5: 103.
- KIM, S. K., and D'APPOLONIA, B. L. 1977. The role of wheat flour constituents in bread staling. The Bakers Digest 51(1): 38.
- KIRSOP, B. H. and BROWN, M. L. 1972. Some effect of wort composition on the rate and extent of fermentation by brewing yeasts. J. Inst. Brew. 78: 51.
- KLOPFENSTEIN, T., and ABRAMS, S. T. 1981. Distillers by-products
 use a review. Nebraska Cattle Day Report. EC 81-218: 2.
- KNAPPERT, D., GRETHLEIN, H., and CONVERSE, A. 1980. Partial acid hydrolysis of cellulosic materials as a pretreatment for enzymatic hydrolysis. Biotech. Bioeng. 22: 1449.
- KOCH, R. B., SMITH, F., and GEDDES, W. F. 1954. The fate of sugars in bread doughs and synthetic nutrient solutions undergoing fermentation with baker's yeast. Cereal Cnem. 31:55

- KULP, K., CHUNG, H., MARTINEZ-ANAYA, M. A., and DOERRY, W. 1985.
 Fermentation of water ferments and bread quality. Cereal Chem.
 62(1): 55
- LYONS, T. P. 1983. Ethanol production in developed countries. Process Biochem. 18(2): 18.
- MAISCH, W. F., SOHOLOY, M., and PETRICOLA, A. J. 1979. Distilled beverages. in: Microbial Technology. Vol II. H. J. Peppler and D. Perlman eds. Academic press, NY.
- MARTIN, C. R. 1981. Characterization of grain dust properties. Trans. ASAE. 24(3): 738.
- MARTIN, C. R. and SAUER, D. B. 1976. Physical and biological characteristics of grain dust. Trans. ASAE. 19(4): 720.
- MARTIN, C. R. and STEPHENS, L. E. 1977. Broken corn and dust generated during repeated handling. Trans. ASAE 20(1): 168.
- MILLER, B. S. 1981. Grain dust What can be done about it? Cereal Foods World 26(5): 239.
- NAFC 1980a. Energy balances in the production and end-use of alcohols derived from biomass. U.S. National Alcohol Fuels Commission. Washington, D.C.
- NAFC 1980b. Farm and cooperative alcohol plant study. U.S. National Alcohol Fuels Commission. Contract No. T16076549. Washington, D.C.
- NAFC 1980c. Fuel alcohol on the farm: A primer on production and use. U.S. National Alcohol Fuels Commission. Washington, D.C.
- NAFC 1981. Fuel alcohol. An energy alternative for the 1980s. Final Report. U.S. National Alcohol Fuels Commission. Washington, D.C.
- NAFFA 1979. A learning guide for alcohol fuel production. National Alcohol Fuel Producers Association. Colby Community College. Lincoln, Nebraska.
- NELLIS, M. 1979. Makin' it on the farm. American Agriculcure News. Iredell, TX.
- NRC 1981. Feeding value of ethanol production by-products. National Academy of Sciences - National Research Council. Washington, D.C.

- OTA 1981. Energy from biological processes. Tecnnical and Envirnmental Analysis. Office of Technicology Assessment. Ballinger Energy Series. Ballinger Publishing Co., A subsidiary of Harper & Row, Publishers, Inc., Cambridge, Massachusetts.
- ROTHMAN, H., GREENSHIELDS, R., and CALLE, F.R. 1983. Energy from alcohol. The Brazilian experience. The University Press of Kentucky, Lexington, Kentucky.
- RUBENTHALER, G. L., FINNEY, P. L., DEMARAY, D. E., and FINNEY, K. F. 1980. Gasograph: design, construction, and reproducibility of a sensitive 12-channel gas recording instrument. Cereal Chem. 57: 212.
- SCHELLER, W. A. 1974. Agricultural alcohol in automotive fuel -Nebraska Gasohol Proc. 8th Nat'l Conference on wheat util. Res. USDA Pub. ARS-W19.
- SCHELLER, W. A. 1981. Gasohol: the U.S. experience. p 633 in: Cereals. A Renewable Resource. Theory and Practice. Y. Pomeranz and L. Munck, eds. The American Association of Cereal Chemists, Inc., St. Paul, MN.
- SCHELLER, W. A. and MOHR, B. J. 1976. Net energy analysis of ethanol production. Amer. Chem. Soc. Div. Fuel Chem. Prpts. 21(2): 29.
- SEBREE, B. R., CHUNG, D. S., and SEIB, P. A. 1983. Brewers' condensed solubles. I. Composition and physical properties. Cereal Chem. 60: 147.
- SERI 1980. Fuel from farms A guide to small-scale ethanol production. SERI/SP-451-519. The National Technical Information Service. Solar Energy Research Institute. Springfield, /irginia.
- SILVA, J. G., SERRA, G. E., MOREITA, J. R., CONCALVES, J. C., and GOLDENBERG, J. 1978. Energy balance for ethyl alcohol production from crops. Science 201(8): 903.
- STARK, W. H. 1954. Chap. 2. Alcoholic fermentation of grain. p 17 in: Industrial Fermentation, Vol I. L. A. Underkofler and R. J. Hickey, eds. Chemical Publishing Co., Inc. New York, NY.
- STOUT, B. A., PEART, R. M., BUCHELE, W. F., and FINCH, E. 1978. Brazil promotes PROALCOOL for petroleum independence. Agr. Eng. 59: 30.

- TITUS, T. C. 1980. Making on-farm ethanol fuel. Fermentation Chemistry and Microbiology. Extension Service, Clemson University, South Carolina.
- TSAO, G. T. 1985. Structures of cellulosic materials and their hydrolysis by enzymes. p 297 in: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck eds. Elsevier Sciences Publishers, Amsterdam, Netherlands.
- USDA 1985a. Feed, outlook and situation report. FDS-297. Economic Research Service. U.S. Department of Agriculture.
- USDA 1985b. Feed, outlook and situation report. FDS-298. Economic Research Service. U.S. Department of Agriculture.
- WU, Y. V., SEXSON, K. R., and LAGODA, A. A. 1984. Protein-rich residue from wheat alcohol distillation: fractionation and characterization. Cereal Chem. 61(5): 423.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. D. S. Chung, major professor, Department of Agricultural Engineering, for his guidance and assistance during the research and in the preparation of this manuscript.

Gratitude is also expressed to Dr. P. A. Seib for providing the facilities and equipment necessary to conduct this research.

Appreciation is extended to Dr. L. E. Erickson and Dr. R. F. Nassar for serving on the advisory committee; and to Dr. O. K. Chung for reviewing the manuscript.

Dr. K. M. Chung is to be thanked for his help in the performance of numerous experiments for this study.

The author wishes to express his very special thanks to his wife for all her encouragement and moral support throughout the course of this work.

ETHANOL PRODUCTION FROM GRAIN DUSTS, BREAD WASTE, AND CAKE WASTE WITH AND WITHOUT BREMERS' CONDENSED SOLUBLES (3CS)

by

CHUL-HO CHOI

B.E. Han-Yang University, Korea, 1980

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

in

FOOD SCIENCE

Department of Agricultural Engineering
KANSAS STATE UNIVERSITY
Mannattan, Kansas

AB STRACT

Alcohol fermentation was performed on bread waste, cake waste, and grain dusts from wheat-corn, corn-sorghum, and sorghum-soybean. Each sample was consecutively saccharified using alpha-amylase and glucoamylase before fermentation. A 1:1 mixture of glucoamylase treated brewers' condensed solubles (3CS) and each of these samples was also fermented to determine whether the mixture increased the rate of fermentation and the yield of ethanol. Distiller's active dry yeast was used at 30°C. The gasograph was used to determine the optimum fermentation times. The yields of ethanol and total fermentable sugars were determined with HPLC.

When equal amounts of BCS (as dry solids) were added, the optimum fermentation times were not affected for grain dusts, but were reduced from 62 hr to 34 hr and from 76 hr to 35 hr on bread waste and cake waste, respectively. Because BCS was a very rich source of available nitrogen, fermentation time was reduced by adding BCS when the substrate was low in nitrogen.

Addition of BCS to grain dusts increased the yields of ethanol from 164-263 ml/kg ds to 305-353 ml/kg ds. But adding BCS only slightly increased the ethanol yields from bakery wastes. When grain by-products had low starch contents, addition of BCS increased the yields of ethanol due to the high available carbon in BCS.

Compared to using grain as a feedstock, ethanol production from grain dust cost only half as much, and ethanol from bakery waste cost three-fourths as much. Adding BCS to grains decreased the ethanol production cost by 25-30%. The cost was also decreased by 20% when BCS was added to bakery waste. The cost was only slightly decreased when BCS was added to grain dust. This result might be due to the relatively high price of BCS compared to that of grain dust. However, more cost benefit would be observed if the cost estimation had included the higher productivity and better by-product credit resulting from adding BCS.